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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/082,034	02/21/2002	William Hildebrand	6680.040	9571

7590 05/03/2004

Dunlap, Codding & Rogers, P.C.
Suite 420
9400 North Broadway
Oklahoma City, OK 73114

EXAMINER	
SMITH, CAROLYN L	

ART UNIT	PAPER NUMBER
1631	

DATE MAILED: 05/03/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/082,034	HILDEBRAND ET AL.	
	Examiner	Art Unit	
	Carolyn L Smith	1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12/5/03*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *3-11* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3-11* is/are rejected.

7)☒ Claim(s) *8* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *07182003, 12052003*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Applicants' election without traverse of Group III (claims 3-5), filed 12/5/03, is acknowledged. Cancelled claims 1-2 and new claims 6-11 are acknowledged.

The information disclosure statements, filed 7/18/03 and 12/5/03, have been fully considered.

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR § 1.821 (a)(1) and (a)(2). See for example, page 41 (second paragraph), 50 (line 23), 51 (lines 4, 6, 14), 52 (last 2 lines), 56 (lines 15 and 16), 66 (line 3), and Figures 5, 6, 8, and 10. However, this application fails to comply with the requirements of 37 CFR § 1.821 through 1.825, because SEQ ID Nos cited along with each sequence in the specification or Figures. Applicants are also reminded that SEQ ID Nos are not required in Figures per se, however, the corresponding SEQ ID Nos then are required in the Brief Description of the Drawings section in the specification. Applicants are also reminded that a CD-ROM sequence listing submission may replace the paper and computer readable form sequence listing copies. Applicant(s) are required to submit a new computer readable form sequence listing, a paper copy, or CD-ROM for the specification, statements under 37 CFR § 1.821 (f) and (g), if there is a need to list additional sequences in the sequence listing. Applicant(s) are given the same response time regarding this failure to comply as that set forth to respond to this office action. Failure to respond to this requirement may result in abandonment of the instant application or a notice of a failure to fully respond to this Office Action.

Claims herein under examination are 3-11.

Specification

The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code, such as on page 2, line 19; page 79, lines 4-5; page 87, line 23; and in Figure 12. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01.

Claim Objections

Claim 8 is objected to because of the following minor informality: "soluable" on line 3 is misspelled. Appropriate correction is requested.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 3-11 are rejected under 35 U.S.C. 101 because the claims are directed to non-statutory subject matter.

These claims are directed to a database which is non-functional descriptive material that includes a compilation or mere arrangement of data (see MPEP § 2106 IV(B)(1) and § 2106 IV(B)(1)(b)). MPEP § 2106 IV(B)(1) states the following regarding non-statutory subject matter:

> Compare In re Lowry, 32 F.3d 1579, 1583-84, 32 USPQ2d 1031, 1035 (Fed. Cir. 1994) (claim to data structure stored on a computer readable medium that increases computer efficiency held statutory) and Warmerdam, 33 F.3d at 1360-61, 31 USPQ2d

> at 1759 (claim to computer having a specific data structure stored in memory held statutory product-by-process claim) with Warmerdam, 33 F.3d at 1361, 31 USPQ2d at 1760 (claim to a data structure per se held nonstatutory). When nonfunctional descriptive material is recorded on some computer-readable medium, it is not statutory since no requisite functionality is present to satisfy the practical application requirement. Merely claiming nonfunctional descriptive material stored in a computer-readable medium does not make it statutory. Such a result would exalt form over substance.
>
> MPEP § 2106 IV(B)(1)(b) states the following regarding non-statutory subject matter:
>
> Descriptive material that cannot exhibit any functional interrelationship with the way in which computing processes are performed does not constitute a statutory process, machine, manufacture or composition of matter and should be rejected under 35 U.S.C. 101.
>
> Where certain types of descriptive material, such as music, literature, art, photographs and mere arrangements or compilations of facts or data, are merely stored so as to be read or outputted by a computer without creating any functional interrelationship, either as part of the stored data or as part of the computing processes performed by the computer, then such descriptive material alone does not impart functionality either to the data as so structured, or to the computer. Such "descriptive material" is not a process, machine, manufacture or composition of matter. (Data consists of facts, which become information when they are seen in context and convey meaning to people. Computers process data without any understanding of what that data represents. Computer Dictionary 210 (Microsoft Press, 2d ed. 1994).)

Claims 3-11 are rejected under 35 U.S.C. 101 because the claims are directed to non-statutory subject matter. As written, claims 3-11 encompass a computer-related invention (database) that appears to lack any physical result performed outside of a computer.

As stated in MPEP § 2106, (IV)(B)(2)(b), to be statutory, a claimed computer-related process must either: (A) result in a physical transformation outside the computer for which a practical application in the technological arts is either disclosed in the specification or would have been known to a skilled artisan (discussed in MPEP § 2106 (IV)(B)(2)(b)(i)), or (B) be

limited to a practical application within the technological arts (discussed in MPEP § 2106 (IV)(B)(2)(b)(ii)).

As stated in MPEP § 2106 (IV)(B)(2)(b)(i), the independent physical acts may be post- or pre-computer processing activity as described below:

> A process is statutory if it requires physical acts to be performed outside the computer independent of and following the steps to be performed by a programmed computer, where those acts involve the manipulation of tangible physical objects and result in the object having a different physical attribute or structure. Diamond v. Diehr, 450 U.S. at 187, 209 USPQ at 8. Thus, if a process claim includes one or more post-computer process steps that result in a physical transformation outside the computer (beyond merely conveying the direct result of the computer operation), the claim is clearly statutory.

> Another statutory process is one that requires the measurements of physical objects or activities to be transformed outside of the computer into computer data (In re Gelnovatch, 595 F.2d 32, 41 n.7, 201 USPQ 136, 145 n.7 (CCPA 1979) (data-gathering step did not measure physical phenomenon); Arrhythmia, 958 F.2d at 1056, 22 USPQ2d at 1036), where the data comprises signals corresponding to physical objects or activities external to the computer system, and where the process causes a physical transformation of the signals which are intangible representations of the physical objects or activities. Schrader, 22 F.3d at 294, 30 USPQ2d at 1459 citing with approval Arrhythmia, 958 F.2d at 1058-59, 22 USPQ2d at 1037-38; Abele, 684 F.2d at 909, 214 USPQ at 688; In re Taner, 681 F.2d 787, 790, 214 USPQ 678, 681 (CCPA 1982).

As stated in MPEP § 2106 (IV)(B)(2)(b)(ii), the computer-related process may be limited to a practical application in the technological arts as described below:

> There is always some form of physical transformation within a computer because a computer acts on signals and transforms them during its operation and changes the state of its components during the execution of a process. Even though such a physical transformation occurs within a computer, such activity is not determinative of whether the process is statutory because such transformation alone does not distinguish a statutory computer process from a nonstatutory computer process. What is determinative is not how the computer performs the process, but what the computer does to achieve a practical application. See Arrhythmia, 958 F.2d at 1057, 22 USPQ2d at 1036.

Claims 3-11 do not fulfill either of these statutory requirements and are therefore rejected under 35 U.S.C. 101 because the claims are directed to non-statutory subject matter.

Claims 3-11 are rejected under 35 U.S.C. 101 because the claims are directed to non-statutory subject matter. As written, the claims appear to be directed to a database that is involved in the manipulation of numbers, abstract concepts or ideas, or signals representing any of the foregoing.

As stated in MPEP § 2106, (IV)(B)(1), if the "acts" of a claimed process manipulate only numbers, abstract concepts or ideas, or signals representing any of the foregoing, the acts are not being applied to appropriate subject matter. Schrader, 22 F.3d at 294-95, 30 USPQ2d at 1458-59. Thus, a process consisting solely of mathematical operations, i.e., converting one set of numbers into another set of numbers, does not manipulate appropriate subject matter and thus cannot constitute a statutory process.

In practical terms, claims define nonstatutory processes if they:

> – consist solely of mathematical operations without some claimed practical application (i.e., executing a "mathematical algorithm"); or
> – simply manipulate abstract ideas, e.g., a bid (Schrader, 22 F.3d at 293-94, 30 USPQ2d at 1458-59) or a bubble hierarchy (Warmerdam, 33 F.3d at 1360, 31 USPQ2d at 1759), without some claimed practical application.

Claims 3-11 do not fulfill any of these statutory requirements and are therefore rejected under 35 U.S.C. 101 because the claims are directed to non-statutory subject matter.

Claims Rejected Under 35 U.S.C. § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3-11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

Claims 3-11 are vague and indefinite due to the unclarity of citing an abbreviation, such as HLA. Correction is suggested by amending in of the full name in parentheses.

Claim Rejections – 35 USC §102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 3-7 are rejected under 35 U.S.C. 102(b) as being anticipated by Schafer et al. (Vaccine, Vol. 16, No. 19, 1998, pages 1880-1884).

Schafer et al. disclose a computer-driven analysis of sequences to permit the identification of peptides that bind to major histocompatibility (MHC) molecules (MHC ligands) such as human leucocyte antigens (HLA) ligands (abstract and page 1883, col. 1, second and third paragraphs). Schafer et al. disclose using a spreadsheet (database) of putative ligands (page

1881, col. 2, third paragraph) and show a list of ligands after binding assays with the HLA (Table 1). Schafer et al. disclose performing in vitro peptide binding assays to assess peptide binding and stability to HLA-B27 and HLA-A2 (page 1881, col. 2, fourth paragraph). Schafer et al. disclose the isolates of ligands as well as their resulting sequences (Table 1, columns 2 and 9). Schafer et al. disclose estimated binding probabilities and highest fold changes in Table 1 (columns 3-6) which represent a linear manipulation of the HLA ligand data, as stated in instant claim 4. Schafer et al. disclose using EpiMatrix, a predictive algorithm to identify MHC ligands (page 1880, col. 2, first paragraph), as stated in instant claim 5. The spreadsheet and computer-driven analysis, as well as binding results described above represent a memory media that stores the data structure of HLA ligand data, as stated in instant claim 6.

Thus, Schafer et al. anticipate the limitations in claims 3-7.

Claim Rejections – 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point the inventor

and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. (e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 3-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schafer et al. (Vaccine, Vol. 16, No. 19, 1998, pages 1880-1884) in view of Thalhammer-Reyero (P/N 5,980,096).

Schafer et al. describe a computer-driven analysis of sequences to permit the identification of peptides that bind to major histocompatibility (MHC) molecules (MHC ligands) such as human leucocyte antigens (HLA) ligands (abstract and page 1883, col. 1, second and third paragraphs). Schafer et al. describe using a spreadsheet (database) of putative ligands (page 1881, col. 2, third paragraph) and show a list of ligands after binding assays with the HLA (Table 1). Schafer et al. describe performing in vitro peptide binding assays to assess peptide binding and stability to HLA-B27 and HLA-A2 (page 1881, col. 2, fourth paragraph). Schafer et al. describe the isolates of ligands as well as their resulting sequences (Table 1, columns 2 and 9). Schafer et al. describe estimated binding probabilities and highest fold changes in Table 1 (columns 3-6) which represent linear manipulations of the HLA ligand data, as stated in instant claim 4. Schafer et al. describe using EpiMatrix, a predictive algorithm to identify MHC ligands (page 1880, col. 2, first paragraph), as stated in instant claim 5. The spreadsheet and computer-driven analysis, as well as binding results described above represent a memory media that stores the data structure of HLA ligand data, as stated in instant claim 6. Schafer et al. do not describe instructions for receiving a soluble HLA ligand data request from a requestor, receiving and

returning a match request, as stated in instant claim 8. Schafer et al. do not describe receiving a request from a remote connection, as stated in instant claim 9.

Thalhammer-Reyero describes an integrated computer-based interface, methods, and systems for the development and deployment of graphical information storage (databases) and retrieval (abstract and col. 4, lines 65-67). Thalhammer-Reyero describes information and mathematical models in the form of tables wherein various forms of information can be extracted from predefined queries (abstract). Thalhammer-Reyero describes a "user" (requestor) as a person that extracts accumulated knowledge and runs simulations (col. 13, lines 47-49). Thalhammer-Reyero describes providing data including DNA and ligands as well as processes and interactions (col. 15, lines 17-34). Thalhammer-Reyero describe a ligand database as seen in Figure 10. Thalhammer-Reyero describes a request, finding matches, and then displaying the list as output (col. 99, lines 36-50). Thalhammer-Reyero describes databases (col. 2, line 17) and using models for prediction and hypothesis formation (col. 3, lines 1-14). Thalhammer-Reyero describes knowledge-based and model-based systems (col. 3, line 36) including inference engines, simulators, user-interfaces to search for and locate information which can be saved and shared with networked remote CPUs or terminals (col. 4, lines 31-56).

Schafer et al. state the need for an effective vaccine against HIV-1 that takes into consideration the variability of HIV strains remains urgent (page 1880, col. 1, first paragraph). Schafer et al. state that EpiMatrix and other computer-driven algorithms that predict MHC ligands place the prospective design of a novel HIV-1 vaccine within reach. One of ordinary skill in the art would have been motivated to use a system can be used by scientists as a new form of interactive research tool to integrate information and data that can be modified and its

parameters adjusted as new information and data pertinent to the system under study becomes available (col. 10, lines 40-45), as stated by Thalhammer-Reyero, in HIV research in order to utilize computer-driven methods of identifying potential leads for HIV-1 vaccine development and put such development within reach (page 1883, col. 2, second paragraph). Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the interactive system using remote requestors, as stated by Thalhammer-Reyero, in the predictive algorithm and HLA ligand database, as stated by Schafer et al., in order to come up with prospectively designed vaccines to variable HIV-1 strains (Schafer et al., page 1880, col. 1, first paragraph and col. 2, second paragraph) where the information is constantly evolving (Thalhammer-Reyero).

Thus, Schafer et al., in view of Thalhammer-Reyero, motivate claims 3-11.

Conclusion

No claim is allowed.

Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993) (See 37 CFR §1.6(d)). The CM1 Fax Center number is (703) 872-9306.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carolyn Smith, whose telephone number is (571) 272-0721. The examiner can normally be reached Monday through Thursday from 8 A.M. to 6:30 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Woodward, can be reached on (571) 272-0722.

Any inquiry of a general nature or relating to the status of this application should be directed to Legal Instruments Examiner Tina Plunkett whose telephone number is (571) 272-0549.

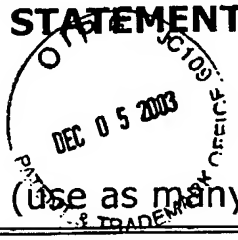
4/14/04

Ardin H. Marschel 4/29/04
ARDIN H. MARSCHEL
PRIMARY EXAMINER

Express Mail: EL 964151056 US
Date Deposited: December 5, 2003

Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT



(use as many sheets as necessary)

Complete if Known	
Application Number	10/082,034
Filing Date	02/21/2002
First Named Inventor	William H. Hildebrand, et al.
Group Art Unit	1631
Examiner Name	C. Smith
Attorney Docket Number	6680.040

U. S. PATENT DOCUMENTS

EXAM INIT.	Cite No. 1	U.S. PATENT NUMBER Number	Kind Code[2] (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
CS	1	4,683,202		Mullis	7/28/1987	
	2	5,256,541		Pouletty et al.	10/26/1993	
	3	5,270,169		Chang et al.	12/14/1993	
	4	5,292,641		Pouletty	3/8/1994	
	5	5,482,841		Buelow	1/09/1996	
	6	5,710,248		Grose	1/20/1998	
	7	5,750,367		Chan	5/12/1998	
	8	5,776,746		Denney, Jr.	7/7/1998	
	9	5,798,209		Chan	8/25/1998	
	10	6,001,365		Peterson et al.	12/14/1999	
✓	11	6,255,073		Cai et al.	7/3/2001	

FOREIGN PATENT DOCUMENTS

EXAM INIT.	Cite No. 1	Foreign Patent Document Office 3	Number 4	Kind Code5 (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
CS	A		WO 95/11702			5/4/1995		
CS	B		WO 97/46256			12/11/1997		
CS	C		WO 98/06749			2/19/1998		
CS	D		WO 00/23053			4/27/2000		

U.S. and Foreign: [1] Unique citation designation number. [2] See attached Kinds of U.S. Patent Documents. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard St.3). [4] Form Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard St. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

Cindy S 7/14/04

NON PATENT DOCUMENTS

EXAM INIT.		Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published
CS	AA	"MOLECULAR CLONING A LABORATORY MANUAL", Maniatis et al., Selected Text "RNA -Dependent DNA Polymerase" p.129, "Isolation of mRNA from Mammalian Cells" pp. 191-193, Cold Harbor Spring Laboratory (1982).
	BB	"LARGE SCALE PRODUCTION OF MURINE MONOCLONAL ANTIBODIES USING HOLLOW FIBER BIOREACTORS", Evans et al., BioTechniques, 6(8):763-767 (1988).
	CC	"HIV-1 REVERSE TRANSCRIPTASE IS A TARGET FOR CYTOTOXIC T LYMPHOCYTES IN INFECTED INDIVIDUALS", Walker et al., Science, 240(4848):64-66 (1988).
	DD	"ASSEMBLY OF MHC CLASS I MOLECULES ANALYZED IN VITRO", Townsend et al., Cell, 62(6):285-295 (1990).
	EE	"ALLELE-SPECIFIC MOTIFS REVEALED BY SEQUENCING OF SELF-PEPTIDES ELUTED FROM MHC MOLECULES", Falk et al., Nature, 351(6324):290-296, (1991).
	FF	"CHARACTERIZATION OF PEPTIDES BOUND TO THE CLASS I MHC MOLECULE HLA-A2.1 BY MASS SPECTROMETRY", Hunt et al., Science, 255(5049):1261-1263 (1992).
	GG	"PEPTIDE BINDING TO HLA-A2 AND HLA-B27 ISOLATED FROM ESCHERICHIA COLI", Parker et al., The Journal of Biological Chemistry, 267(8):5451-5459 (1992).
	HH	"ENDOGENOUS PEPTIDES OF SOLUBLE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I MOLECULE, H-2Lds: SEQUENCE MOTIF, QUANTITATIVE BINDING, AND MOLECULAR MODELING OF THE COMPLEX", Corr et al., J. Exp. Med., 176(6):1681-1692 (1992).
	II	"THE SPECIFICITY AND EFFICIENCY OF ENDOGENOUS PEPTIDES IN THE INDUCTION OF HLA CLASS I ALPHA CHAIN REFOLDING", Tanigaki, Eur J. Immunol., 22(8):2177-2180 (1992).
	JJ	"CAN ONE PREDICT ANTIGENIC PEPTIDES FOR MHC CLASS I-RESTRICTED CYTOTOXIC T LYMPHOCYTES USEFUL FOR VACCINATION?", Calin-Laurens et al., Vaccine, 11(9): 974-978 (1993).
	KK	"DIRECT IDENTIFICATION OF AN ENDOGENOUS PEPTIDE RECOGNIZED BY MULTIPLE HLA-A2.1-SPECIFIC CYTOTOXIC T CELLS", Henderson et al., Proc. Natl. Acad. Sci. USA, 90:10275-10279 (1993).
	LL	"CHARACTERIZATION OF ENDOGENOUS PEPTIDES ELUTED FROM THE CLASS I MHC MOLECULE HLA-B7 DETERMINED BY MASS SPECTROMETRY AND COMPUTER MODELING", Huczko et al., J. Immunol., 151(5):2572-2587 (1993).
	MM	"FLOW-CYTOMETRIC DETERMINATION OF PEPTIDE-CLASS I COMPLEX FORMATION IDENTIFICATION OF p53 PEPTIDES THAT BIND TO HLA-A2", Zeh et al., Human Immunology, 39:79-86 (1994).
	NN	"PEPTIDE BINDING TO THE MOST FREQUENT HLA-A CLASS I ALLELES MEASURED BY QUANTITATIVE MOLECULAR BINDING ASSAYS", Sette et al., Molecular Immunology, 31(11): 813-822 (1994).
	OO	"BINDING OF A PEPTIDE ANTIGEN TO MULTIPLE HLA ALLELES ALLOWS DEFINITION OF AN A2-LIKE SUPERTYPE", del Guercio et al., J Immunol., 154(2):685-693 (1995).
	PP	"AN HLA CLASS I PEPTIDE-BINDING ASSAY BASED ON COMPETITION FOR BINDING TO CLASS I MOLECULES ON INTACT HUMAN B CELLS IDENTIFICATION OF CONSERVED HIV-I POLYMERASE PEPTIDES BINDING TO HLA-A*0301", van der Burg et al., Human Immunology, 44:189-198 (1995).
	QQ	"MEASURING INTERACTIONS OF MHC CLASS I MOLECULES USING SURFACE PLASMON RESONANCE", Khilko et al., J. Immunol. Methods, 183(1):77-94 (1995).
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U. S. PATENT DOCUMENTS

EXAM INIT.	Cite No. 1	U.S. PATENT NUMBER Number	Kind Code2 (If known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

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CS	AA	RAMMENSEE HANS-GEORG ET AL: "SYFPEITHI: Database for MHC ligands and peptide motifs" IMMUNOGENETICS, SPRINGER VERLAG, BERLIN, DE, vol. 50, no. 3-4, November 1999 (1999-11), pages 213-219, XP002183663 ISSN: 0093-1177 page 214; figure 1 tables 3, 4 page 213; column 2, line 25 - line 29

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CS	AB	BRUSIC VLADIMIR ET AL: "MHCPEP, a database of MHC-binding peptides: Update 1997" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 26, no. 1, January 1, 1998 (1998-01-07) pages 368-371, XP002204283 ISSN: 0305-1048 figure 1; table 2
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Notice of References Cited	Application/Control No. 10/082,034	Applicant(s)/Patent Under Reexamination HILDEBRAND ET AL.	
	Examiner Carolyn L Smith	Art Unit 1631	Page 1 of 1

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Prediction of well-conserved HIV-1 ligands using a matrix-based algorithm, EpiMatrix

James Robert A. Schafer*, Bill M. Jesdale*, Judith A. George*, Nicola M. Kouttab† and Anne S. De Groot*‡

This preliminary study was undertaken to identify new human leucocyte antigens (HLA) ligands from human immunodeficiency virus type 1 (HIV-1) which are highly conserved across HIV-1 clades and which may serve to induce cross-reactive cytotoxic T lymphocytes (CTLs). EpiMatrix was used to predict putative ligands from HIV-1 for HLA-A2 and HLA-B27. Twenty-six peptides that were both likely to bind and also highly conserved across HIV-1 strains in the Los Alamos HIV sequence database were selected for binding assays using the T2 stabilization assay. Two peptides that were also highly likely to bind (for A2 and B27, as determined by EpiMatrix) and well conserved across HIV-1 strains, and had previously been described to bind in the published literature, were also selected to serve as positive controls for the assays. Ten new major histocompatibility complex (MHC) ligands were identified among the 26 study peptides. The control peptides bound, as expected. These data confirm that EpiMatrix can be used to screen HIV-1 protein sequences for highly conserved regions that are likely to bind to MHC and may prove to be highly conserved HIV-1 CTL epitopes. © 1998 Published by Elsevier Science Ltd. All rights reserved

Keywords: EpiMatrix; HIV-1; ligands; CTL (cytotoxic T cell); clade; algorithm

The need for an effective vaccine against human immunodeficiency virus type 1 (HIV-1) that takes into consideration the variability of HIV strains remains urgent[1]. Whole protein vaccines, live attenuated vaccines, and vaccine vectors containing whole proteins are currently being developed. In attempts to increase the range of HIV strains represented by vaccines in clinical trials, some HIV-1 vaccines have been modified to include HIV protein sequences from diverse HIV-1 strains. These modified whole protein or gene-based vaccines represent one approach to HIV-1 vaccine development.

An alternative approach has been to construct HIV-1 vaccines based on T-cell epitopes in HIV-1 sequences that are conserved across clades. *In vitro* studies of cytotoxic T lymphocytes (CTL) from HIV-1-infected or vaccinated individuals have documented cross-clade recognition of CTL epitopes[2]. Cross-clade CTL recognition may be due to conservation of T-cell epitopes across clades or to the degeneracy of T-cell receptor recognition.

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The prospective design of multivalent HIV immunogens tailored to reflect the diversity of HIV isolates, and to promote cross-clade protection in settings where more than one HIV strain and more than one HIV clade is being transmitted, has been suggested previously[3,4]. This study explored the use of EpiMatrix, a matrix-based algorithm for T-cell epitope prediction, to prospectively identify conserved class I-restricted MHC ligands and potential CTL epitopes. EpiMatrix and other computer-driven algorithms that predict putative MHC ligands and CTL epitopes[5–7] place the prospective design of a novel HIV-1 vaccine with these critically important characteristics within reach.

Such prospectively designed vaccines are based on the central role of CTL in the host immune response to HIV-1, and the understanding that the first step in the search for HIV-1 CTL epitopes may be to identify peptides that bind to the host major histocompatibility complex (MHC). Recognition of such MHC ligands by CTL is dependent on the presentation of the T-cell epitope to the T cells in the context of MHC molecules. Peptides presented in conjunction with class I MHC molecules (to T cells) are derived from foreign or self-protein antigens that have been processed in the cytoplasm[8–10]. The peptides bind to MHC molecules in a linear fashion; the binding is determined by the interaction of the peptide's amino acid side-chains with binding pockets in the MHC molecule. Binding of

peptides to MHC molecules is constrained by the nature of the side-chains; only selected peptides will fit the constraints of any given MHC molecule's binding pockets.

The characteristics of peptides that are likely to bind to a given MHC can be directly deduced from pooled sequencing data (from peptides bulk-eluted off MHC molecules), from MHC binding peptide libraries. The TB/HIV Research Lab has developed a method to describe the relative promotion or inhibition of binding afforded by each position in a peptide to the MHC of interest.

EpiMatrix ranks all 10 amino acid long segments from any protein sequence by estimated probability of binding to a given MHC, by comparing the sequence to a matrix. The estimated binding probability (EBP) is derived by comparing the EpiMatrix score to those of known binders and presumed non-binders. Retrospective studies have demonstrated that EpiMatrix accurately predicts MHC ligands[11,12].

In this study, we implemented EpiMatrix to examine the sequences of HIV-1 strains published on the 1995 version of the Los Alamos National Laboratory HIV Sequence database[13]. We identified conserved regions and then examined these for their potential to bind to one of two MHC alleles (A2 and B27). We prospectively identified conserved MHC ligands which may be useful for HIV-1 vaccine development.

METHODS

Generation of an MHC binding matrix motif

Various methods were used in the generation of MHC binding matrix motifs. Briefly, independent sources of information on the relative promotion or inhibition of each amino acid in each position are identified. For each source of information, an estimation of the relative promotion or inhibition of binding is quantified. In a generic sense, this quantification is based on a relative rate calculation, the rate of an amino acid in a given position relative to its median rate across all positions. These matrix motifs, based on single sources of information (such as a list of known ligands (Roman M. Chicz, personal communication) pooled sequencing of naturally eluted peptides[15], peptide side-chain scanning techniques[6], or the identification of ligands with specific characteristics through random phage techniques), are then combined in a way which attempts to maximize the resultant matrix motif's ability to separate a list of known ligands from the other peptides contained within their original sequences. The two matrix motifs based on single datasets with the best individual predictive power (assessed using the Kruskal–Wallis non-parametric test) are first combined with each other. The best resultant of these two was then combined with the third most individually predictive, and so on. The result of this process was then combined with the method of Parker *et al.*[16] to achieve a final predictive matrix motif for each MHC allele.

Generating an EpiMatrix score

Each putative MHC binding region within a given protein sequence is scored by estimating the relative promotion or inhibition of binding for each amino acid, and summing these to create a summary score for the entire peptide. Higher EpiMatrix scores indicate greater MHC binding potential. After comparing the score to the scores of known MHC ligands, an 'estimated binding probability' or EBP, is estimated. The EBP describes the proportion of peptides with EpiMatrix scores as high or higher that will bind to a given MHC molecule.

EBP is derived from the EpiMatrix score by determining how many published ligands for the allele would earn that same score or a higher score (a measure of sensitivity). EBPs range from 100% (highly likely to bind) to less than 1% (very unlikely to bind). The majority of 10mers in any one protein sequence fall below the 1% estimated binding probability for any given MHC binding matrix.

Selection of peptides

For each protein, env, pol, nef, and tat was analyzed independently. The sequence for each HIV-1 isolate in the Los Alamos HIV sequence database[13] was divided into ten amino acid long strings which overlapped by nine. These 10-mer strings were then compared to the A2 and B7 MHC binding matrix motifs (EpiMatrix version 1.0). Peptides that scored higher than 50% EBP were selected. Each of these putative ligands was compared to all the others using a spreadsheet and command macro which orders the strings from those which are common to many of the sequences to those which were unique (*Figure 1*). Strings that were present in 'more' HIV-1 isolates (the exact number depended on the number of isolates available in the LANL database) were selected for the next phase of the analysis. Twenty-eight peptides were selected using this method. One of the selected peptides corresponded to a published CTL epitope, and was selected to serve as a control. An additional peptide selected to serve as a positive control as for this study, KRWIILGLNK, scored lower on the B27 matrix than 50%, however, it was the only available HIV-1 B27 ligand that had been fine-mapped.

The T2 *in vitro* peptide binding assay was performed as recently described by Nijman *et al.*[17] This assay relies on the ability of exogenously added peptides to stabilize the Class I/beta-2 microglobulin structure on the surface of TAP-defective cell lines[14]. For these experiments, antigen processing mutant cell line T2 transfected with the HLA B27 gene (T2/B27), was kindly provided by P. Cresswell (New Haven, CT, USA). These cells were cultured in Iscove Modified Dulbecco's Medium (IMDM), 10% fetal bovine serum, and 20 ug/ml gentamycin. A monoclonal antibody to HLA-B27 produced by the ATCC HB-119, ME1 hybridoma[18] was used to assess HLA-B27 expression at the cell surface (indicating peptide binding and stabilization of the B27 molecule). The monoclonal antibody produced by the ATCC HB-82, BB7.2 hybridoma[19] was used to assess HLA-A2 expression at the cell surface.

Three hundred thousand cells in 100 μl of IMDM, 10% FBS, and 20 μg/ml gentamycin medium were incubated with no peptide, or 100 μl synthetic peptide solution overnight at 37°C, in an atmosphere of 5% CO_2. The T2 cell/peptide suspension was pelleted at 1000 rpm, the supernatant was discarded, and the

suspension was stained with 100 μl of BB7.2, an HLA-A2-specific mouse monoclonal primary antibody (1 h at 4°C). Two wells per peptide did not receive the primary antibody, but only the PBS staining buffer. The cells were washed three times with cold (4°C) staining buffer PBS, 0.5% FBS, 0.02% NaN_3, and stained for 30 min at 4°C with 100 μl FITC-labelled goat anti-mouse immunoglobulin (Pharmingen, 12064-D). The cells were again washed three times and fixed in 1% paraformaldehyde. Fluorescence of viable T2 cells was measured at 488 nm on a FACScan flow cytometer (Becton-Dickinson, NJ).

A total of 12 wells was assayed per peptide (one well each with peptide at 0, 2, 20 and 200 μg/ml were repeated using primary antibody for the molecule the peptide is predicted to bind to, the primary antibody to the molecule the peptide was not predicted to bind to, and no primary antibody).

Analysis and interpretation of binding assays

Peptide binding to MHC molecules stabilizes MHC expression at the cell surface, and can be measured by FACS sorting the cells. The data produced by the FACS analysis represented the mean linear fluorescence (MLF) of 10000 events. We used a cut-off of 1.3-fold greater MFI in any of the three wells with peptide than the control well as the criterion for positive binding.

RESULTS

Twenty-eight peptides were tested in binding assays. Two of the 28 were previously published ligands. Ten peptides induced an increase in the MFI of 1.3-fold or greater (*Table 1*). The published controls bound as expected.

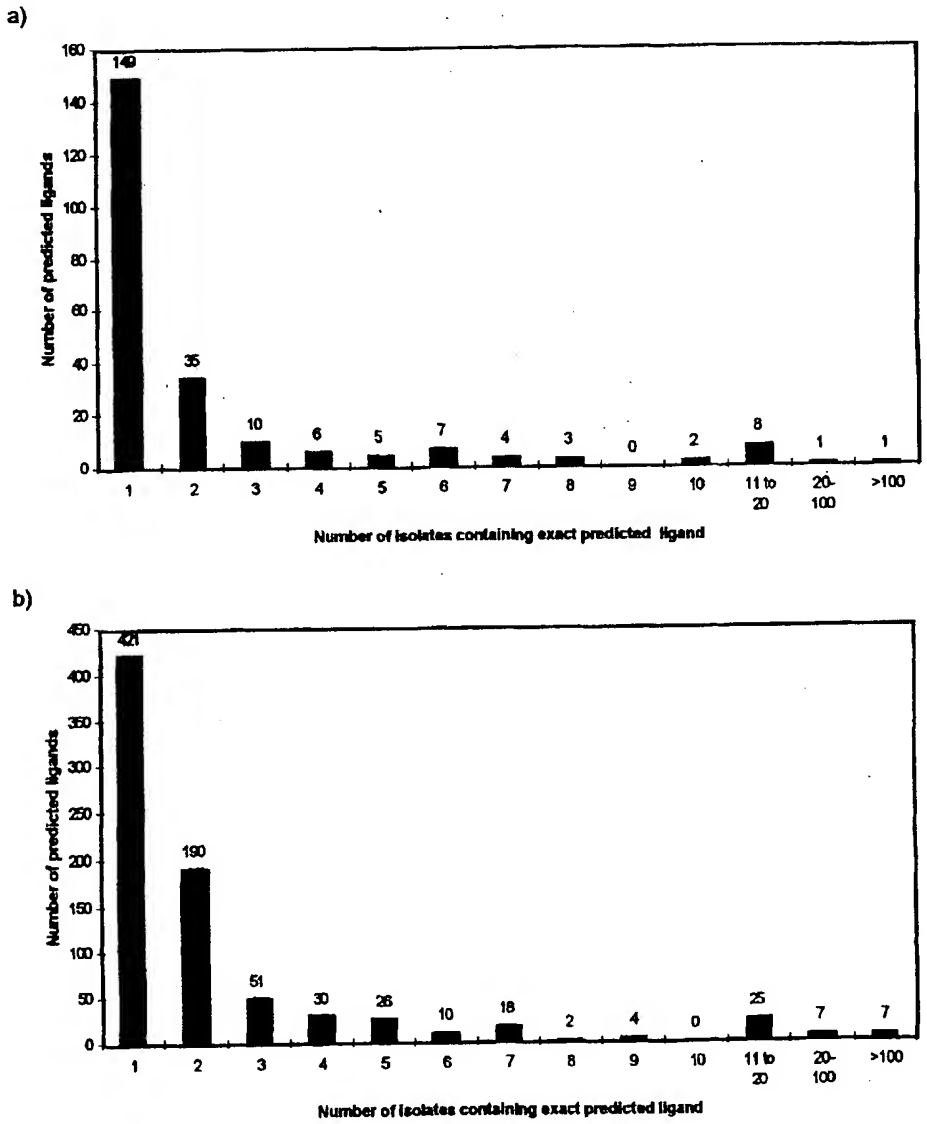

Figure 1 These histograms show the distribution of the number of HIV-1 isolates in which 8- to 11-mer peptides predicted to bind **(A)** HLA-A2; and **(B)** HLA-B27 are exactly conserved

Table 1 Results of binding assays and characteristics of peptides

No.	Sequence	A2 EBP (%)	B27 EBP (%)	A2-fold increase (≥1.3)	B27-fold increase (≥1.3)	Published (region)	Protein	Number of isolates with exact AA sequence	Approximate position in LA	Clade A	B	C	D	E	Other
24	KLTPLCVTLN	55.68	0	1.33	—	No (Yes)	Env	159	gp120–120	X	X	X	X	X	X
25	AEWDRVHPV	66.42	0	1.35	—	No (Yes)	Gag	36	gag-215	X	X		X		X
26	SLFNTVATL	62	0	—	—	No (Yes)	Gag	18	gag-100	X	X	X	X		X
27	ELHPDKWTV	57.03	0	—	—	No (Yes)	RT	17	RT-354	X					
28	GMDDPEREVL	72.52	0	—	—	No (Yes)	Nef	17	nef-170		X				
29	GMDDPEKEVL	87.51	0.01	2.7	—	No (No)	Nef	16	nef-170		X				
30	HLWRWGTMLL	76.69	0	1.33	—	No (No)	Env	10	gp120–30		X		X		X
31	LLLTRDGGVN	55.68	0	—	—	No (No)	Env	<10	gp120–452	X			X		
32	HLWKWSTMLL	90.92	0	1.63	—	No (No)	Env	<10	gp120–20				X		
33	ILKEPVHGV	97.47	0	1.54	—	Yes (Yes)	RT	<10	RT-480		X				
34	KRWILGLNK	0	14.22	—	3.61	Yes (Yes)	Gag	79	gag-263		X				
35	CRIKQIIN	0	99.08	—	—	No (Yes)	Env	185	gp120–420	X	X	X	X		X
36	CRIKQIINMW	0	99.52	—	1.74	No (Yes)	Env	150	gp120–420	X	X	X	X		X
37	VSFEPIPIHF	0.20	55.61	1.45	—	No (No)	Env	109	gp120-215	X	X	X	X		X
38	RCSSNITGL	0.01	62.11	—	—	No (No)	Env	101	gp120–416		X		X		X
39	VSFEPIPIHY	0	98.22	—	—	No (No)	Env	101	gp120–215	X	X	X	X		X
40	CRIKQIVNM	0	91.33	—	—	No (Yes)	Env	75	gp120–420	X	X		X	X	X
41	IRSENITNN	0	82.77	—	—	No (No)	Env	42	gp120–275	X					
42	IRIFIMIV	0.05	89.06	—	—	No (No)	Env	19	gp41–175	X	X	X	X		
43	ISFDPIPIHY	0.01	67.49	—	—	No (No)	Env	15	gp120–215					X	
44	YRTGDIIG	0	56.14	—	—	No (Yes)	Env	15	gp120–330					X	
45	IRIGPGQTFY	0.07	75.36	—	—	No (No)	Env	13			X	X			
46	GCSGKIIC	0	61.09	—	—	No (Yes)	Env	12	gp41–90	X				X	
47	RRRAPQDS	0	67.49	—	—	No (No)	Tat	12			X				
48	IRSENITDN	0	59.28	—	—	No (No)	Env	11	gp120–275				X	X	
49	CRIKQFIN	0	76.92	—	1.53	No (Yes)	Env	<10	gp120–420		X				
50	KRISIGPGR	0	56.93	—	1.78	No (Yes)	Env	<10	gp120–320		X				
51	GCQQIIEQL	0.10	78.95	—	—	No (No)	Env	<10		X					
52	GRRGWEILKY	0.01	59.8	—	3.27	No (Yes)	Env	<10	gp41–270		X				

The 8- to 11-mer peptides synthesized for analysis in this study are shown. The third and fourth columns show the estimated binding probability for peptides with EpiMatrix scores at least as high as these peptides. The fifth and sixth columns give the highest fold-change in MFI at any of three concentrations if over 1.3. The seventh column indicates whether the peptide has been published as a known epitope restricted to the appropriate allele. Parentheses indicate that the peptide is contained within an epitope of unknown restriction. The eighth column indicates the protein of origin. The ninth column indicates the number of isolate sequences containing this exact amino acid sequence. The tenth column indicates the approximate position of this ligand relative to the LAI reference strain. The eleventh through sixteenth columns indicate whether any of the sequences in which the peptide is conserved are designated as belonging to clades A–E, or another clade

Peptides shown here were selected because they were predicted to bind to A2, and not to B27, or vice versa. None of the peptides predicted to bind to A2 bound to B27 and vice versa.

SUMMARY

We performed prospective definition of conserved HIV-1 regions using EpiMatrix version 1.0. Rapid identification of MHC ligands, which can then be tested in T-cell assays, is desirable for HIV-1 vaccine development. Computer-driven analysis of HIV sequences will permit the prospective identification of such conserved CTL epitopes.

Determination of peptides that bind to major histocompatability (MHC) molecules (MHC ligands) can be the first step in the process of identifying T-cell epitopes. Identification of MHC ligands from primary HIV-1 sequences is particularly relevant for HIV vaccine development and immunopathogenesis research. Matrix-based motifs have been developed to improve on the specificity of anchor-based motifs: the advantage of matrix motifs is that peptides can be given a score that represents the sum of the potential for each amino acid in the sequence to promote or inhibit binding.

Predicting regions of immunological interest is only the first step to determining whether the region is likely to be recognized by primed T cells, and to be defined as a CTL epitope. Predictions must be confirmed by binding assays, so as to determine whether a peptide representing that region indeed binds to the MHC for which it was predicted (e.g., T2 cell binding assay). Immunogenicity of the peptides must also be confirmed by measuring whether CTL recognize the peptide in T-cell assays.

Methods of analysis developed in the TB/HIV Research Lab also permit the comparison of putative MHC ligands across HIV-1 clades, and permit the weighting of predictions for the prevalence of HLA alleles in human populations. Utilization of these computer-driven methods will put the prospective identification of cross-clade (cross-reactive) and promiscuous epitopes for HIV-1 vaccine development within reach.

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